Open Access

Phytopathogenomics and Disease Control*., *2024, 3(2)213-220
ISSN (Online):2957-5842; ISSN (Print):2957-5834
DOI: https://doi.org/10.22194/Pdc/3.1023
https://societyfia.org/journal/PDC

Society for Innovative Agriculture

Comparative study of bio-control agents against fusarium wilt in okra caused by *Fusarium oxysporum* pv. *vasinfectum*

Syeda Sana Fatima[1], Muhammad Amjad Ali[1], Muhammad Jabran[2], Muhammad Nabeel Afzal[1], Muhammad Huzaifa Tanveer[1], Shahneeza Aimal Nadeem[1] and Amjad Abbas[1]*

[1]Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan; [2]State Key Laboratory for Biology of Plant Diseases, Institute of Plant Protection, CAAS, 100193, Beijing, China.
*Corresponding author's e-mail: amjad.abbas@uaf.edu.pk

Okra (*Abelmoschus esculentus*) is a widely cultivated vegetable crop that is susceptible to various pathogens, including *Fusarium oxysporum*, which causes severe disease of wilt. Chemical control using fungicides currently poses risks to human and animal health. To find ecofriendly method, the present study aimed to compare the effectiveness of different bio-control agents (BCAs) against Fusarium wilt in Okra for eco-safe management. Okra seeds were taken from Vegetable Research Institute, Ayub Agricultural Research Institute. Disease samples were collected, and the pathogen was isolated, purified, and multiplied by using potato dextrose agar media. Pure culture was used for the isolation of DNA and amplification of the ITS region was accomplished by using ITS-1 and ITS-4 primers. The fragments were visualized by running it on 1% agarose gel. The PCR product of *F. oxysporum pv. vasinfectum* was successfully confirmed based on size of fragment by comparing with 1 kb marker. It was verified that the selected primer pair specifically amplified the anticipated 18S rDNA fragment, measuring 500 base pairs in length. Moreover, *in-vitro* tests revealed that BCAs like *Bacillus spp.* inhibited Fusarium fungus by 41.2%, while *Trichoderma* inhibited it by 38.5%. Control groups exhibited no inhibition of pathogen growth. Taken together, this effort has important implications for the implementation of biological management strategies in Okra field to mitigate the impact of Fusarium wilt disease.

Keywords: Biocontrol, Fusarium wilt, Okra, *Bacillus spp.*, T. harzianum Fusarium wilt, Bio-control agents, eco-friendly management, fusarium oxysporum, fungicides.

INTRODUCTION

Fusarium wilt is a devastating disease caused by the soil-borne fungus *F. oxysporum* pv. *vasinfectum*, which affects a wide range of crops worldwide, including okra (Kumar, 2019). The pathogen infects the root system of plants, causing yellowing, wilting, and eventual death. It is one of the most economically important diseases in okra production, leading to significant yield losses and affecting the quality of the harvested produce (Ashraf *et al.*, 2018). For economic considerations and limited land resources, pressure of produce in agriculture which involves continuous monoculture varieties, has emerged as a significant component of modern agriculture in the present era (Banerjee *et al.*, 2019).

With the progression of intensive agricultural practices, researchers have increasingly acknowledged that the dissemination of soil-borne pathogens aided with accumulation of self-toxic compounds, imbalance in nutrient availability, and degradation of soil physicochemical properties are the primary factors responsible for the difficulties encountered in successive cropping (Xie *et al.*, 2017). Therefore, growth of severe soil-borne pathogens which have become a main problem to agricultural production worldwide (Ma *et al.*, 2023). The use of chemical fungicides to control Fusarium wilt has been the most common practice in the past. However, the negative impact of agrochemicals on the environment and human health, along with the development of fungicide-resistant strains of the fungus, has led to the search for alternative control measures (Ons *et al.*, 2020).

Therefore, biological control agents, which control plant pests, have emerged as a promising alternative to chemical fungicides (Lahlali *et al.*, 2022). Biological approaches to

Fatima, S.S., M.A. Ali, M. Jabran, M.N. Afzal, M.H. Tanveer, S.A. Nadeem and A. Abbas. 2024. Comparative study of bio-control agents against fusarium wilt in okra caused by *Fusarium oxysporum* pv. *Vasinfectum* . Phytopathogenomics and Disease Control 3:213-220.
[Received 11 Jan 2024; Accepted 14 May 2024; Published 18 Jun 2024]

combat the occurrence of soil-borne diseases are very feasible and environmentally friendly which have been used successfully (Kipngeno *et al.,* 2015). This study provides a comprehensive overview of biological control agents, their modes of action, and their mechanisms specifically targeting *F. oxysporum pv. vasinfectum* pathogen. Research focused on *Bacillus spp.* and *Trichoderma harzianum* species as biocontrol agents have indicated that prior screening of BCA in the lab is important.

However, it is evident that biological control holds an advantage over other management practices. Bio-control agents have developed diverse strategies to suppress pathogens, including competing for resources, producing antimicrobial substances, engaging in parasitism inducing plant defense responses, and metabolizing germination stimulants (Lahlali *et al.,* 2022). Hence, the current study assessed the efficacy of biological control agents in controlling the disease after necessary molecular identification of the pathogen. The result of this study provides valuable insights into the effectiveness of bio-control agents and their potential as an eco-friendly alternative to chemical fungicides for controlling Fusarium wilt in Okra.

MATERIALS AND METHODS

The experiment was conducted in earthen pots and commonly grown cultivar of okra, Sabz pari, and a hybrid were used in the study. The okra seeds were collected from Vegetable Research Institute, Ayub Agricultural Research Institute (AARI), Faisalabad. The seeds were sown in pots in the greenhouse of the Department of Plant Pathology, UAF, and the germination of seeds was regularly checked. Two BCA treatments, positive and negative controls were used, and three replications of each treatment and control were maintained for accuracy. In each replication, there were 32 pots. Prior to pot experiment diseased samples were collected for pathogen isolation and characterization from different regions of district Faisalabad.

***Isolation and purification of F. oxysporum pv. Vasinfectum*:** PDA media were used for the isolation and purification of fungal pathogen associated with wilt disease in okra. The media was sterilized at 121ºC at 15psi pressure for 15 min. To inhibit bacterial growth 50 mg/L kanamycin antibiotic was added in the media before setting. The preparation of PDA media involved boiling 200g of potatoes in distilled water for 10 minutes to extract potato starch. The starch infusion was sieved and mixed with other ingredients in a sterile bottle with 1L of distilled water. The media was cooled and poured into sterile petri plates in a laminar flow chamber, with approximately 20mL of media per plate. The plates were then solidified in the laminar flow chamber. Plates were kept for 10-14 days for full growth in the incubator at 25±2ºC. Fungal growth was then scratched off using a spatula, and spores were collected from the culture by using cold water treatment. These spores were passed through muslin cloth to separate them from mycelia, ensuring pure spore suspension. Confirmation of the spores' purity was done through microscopic studies. To further analyze the pathogen, slides were prepared after mass culturing. Spore counts were adjusted to 2×10^6 spores/ml after calculating with Hemocytometer. A clean glass slide with a drop of distilled water was used, and single hyphae from the purified culture plate were mixed with the water using a needle. The pathogen's characteristic features, such as septation of mycelia, spore morphology, and shape, were observed under different magnification powers using a microscope. The identification of the pathogen as *F. oxysporum* pv. *vasinfectum*, associated with wilt disease, was based on available literature on morphology, including colony color and size, spore shape, structure, and growth pattern. To further characterize the pathogen and determine its physiological peculiarities, isolated spores were stained using trypan blue dye. The staining process involved adding the dye to the spore solution on a glass slide. Trypton blue dye was employed to stain the spores. Stain was added to the spore solution. About 10 µl of spore's suspension was taken on a glass slide and equal volume of working solution of trypan blue dye was added to the spore mass.

***Preparation of liquid broth (LB) media*:** Biocontrol bacteria like *Bacillus spp.* having maximum growth inhibition properties against fusarium pathogen. The bacteria were grown in test tube for dual plate assay under *in-vitro* conditions. For culturing of bacteria LB medium was prepared which is a common bacterial culture medium after autoclaving. For one liter of LB broth media 10g tryptone, 5g yeast extract, and 10 NaCl were used. While for LB agar media, 15g agar was added per liter. After all formulations of make the volume up to mark, the media were autoclaved. For culturing bacteria in suspension LB broth media was used. *T. harzianum* were grown on the PDA media and plugs were cut from the mature culture for dual plate assays.

***In-vitro screening of biocontrol agents under controlled conditions*:** For the biological management trials were conducted in pots, plants were grown and subjected to different treatments. The BCA (biological control agent) was applied to the plants through spraying and drenching methods. Each treatment consisted of eight pots. Throughout the trial, proper agricultural practices such as irrigation, hoeing, and weeding were diligently carried out. The extent of disease was measured using a disease measuring scale.

Bio-control agents (BCAs) obtained from Department of Soil and Environmental Sciences, University of Agriculture, Faisalabad. Dual culture plate assays were used for this purpose. *T. harzianum* was used as a control and was placed on one side of the petri plates containing potato dextrose agar (PDA) while the pathogen was on the other side. During the antagonistic test, the fungal pathogen Fusarium was placed

opposite to the BCA to assess their interaction and antagonistic effects. Incubation of the plates took place at a temperature of 25°±2C, and the inhibition of the pathogen was observed regularly. The growth of the fungal pathogen in response to the BCA was carefully measured, and the data obtained was used to calculate the percentage of inhibition by using control plate of pathogenic growth. BCAs exhibiting the highest inhibition properties were then selected for application in pots, with the objective of managing wilt disease. The experiment was carried out using pots which were divided into four groups, with each group consisting of three replications. The first group was designated as the negative control, where only inoculum was applied. The second group took fungal inoculum followed by the application of *Bacillus spp.* as the BCA after 7 days. In the third group, fungal inoculum was applied followed by the application of *T. harzianum* as the BCA after 7 days. The fourth group was the positive control treatment.

***Disease incidence and disease severity*:** The disease incidence and disease severity were also calculated by following formula (Tucho and Yadessa 2014).

$$\text{Disease Incidence} = \frac{\text{No.of infected plants}}{\text{Total no.of plants}} \times 100$$

$$\text{Disease Severity} = \frac{\text{No.of infected leaves}}{\text{Total no.of leaves}} \times 100$$

The disease rating scale used in this research was based on six grades ranging from 0 to 9. Grade 0 represented plants that were immune to the disease, while grade 1 indicated plants that had a disease incidence of 1-5% and were considered resistant. Plants with a disease incidence of 6-10% were classified as moderately resistant (grade 3), while those with a disease incidence of 11-20% were classified as moderately susceptible (grade 5). Plants with a disease incidence of 21-35% were considered susceptible (grade 7), and those with a disease incidence of 36% and above were classified as highly susceptible (grade 9). The response of each plant to the disease was recorded based on its assigned grade.

***Internal transcribed spacer region (ITS) amplification*:** The *F. oxysporum* pv. *vasinfectum* pathogen was cultured using PDA artificial media on 5 plates. After full growth of the plates, the mycelia and spores were harvested from these plates and standard procedures and protocols were followed to isolate DNA from fungal pathogens (Samarrai and Schmid, 2000). Freshly grown fungal cultures were used for DNA isolation, which involved scraping the fungus with a sterilized glass slide and grinding it with an autoclaved pestle and mortar along with fine sand and lysis buffer. Proteinase K and RNase were added, followed by the addition of K-acetate buffer. The mixture was centrifuged, and the supernatant was collected. Isopropanol was added to the supernatant, and the resulting mixture was centrifuged again to obtain a DNA pellet. After washing with ethanol, the pellet was dried and resuspended in TE buffer.

Agarose gel electrophoresis was then performed to confirm the presence of DNA. A gel was prepared by dissolving agarose in TE buffer, and ethidium bromide was added for visualization. The gel was cast with a comb and allowed to solidify. DNA samples and a DNA marker were loaded into the wells of the gel, and electrophoresis was carried out for the separation of DNA bands. TAE buffer was used for gel electrophoresis, and the gel was visualized under UV light using a transilluminator. PCR was performed using the ITS primer pair for amplifying the 18S ribosomal gene. The PCR mixture was prepared in PCR tubes and subjected to thermal cycling.

The PCR thermocycling conditions were as follows: an initial denaturation step at 98 °C for 2 minutes, followed by 30 cycles of denaturation at 98 °C for 15 seconds, annealing at 55 °C (for bacteria) or 50 °C (for fungi) for 30 seconds, and extension at 72 °C for 30 seconds. A final extension step was performed at 72 °C for 5 minutes. After amplification, PCR products were purified using an AxyPrep DNA Gel Extraction Kit (Axygen, Union City, CA, USA). In the literature, AL-Taae *et al.* (2019) utilized a universal primer set (ITS1 & ITS4) in their PCR-based identification of *F. oxysporum*. The efficiency and quality of the extracted DNA were assessed using 1% agarose gel electrophoresis and quantified using a nanodrop spectrophotometer. The ITS-1 (TCCGTAGGTGAACCTGCGG) forward primer and ITS-4 (TCCGTAGGTGAACCTGCGG) reverse primers targeting the 18S ribosomal gene were designed for amplifying target sequence of DNA in the genome of fungal pathogen.

***Statistical analysis*:** Statistical analysis was performed on the data using Statistix (Ver. 8.1). A completely randomized block design (CRBD) was used for analyzing the disease incidence, disease severity and growth parameters. Treatment means were separated using an LSD test at a significance level of P=0.05.

RESULTS

Isolation and morphological identification of *F. oxysporum pv. Vasinfectum*: The study confirmed the presence of a pathogen which was successfully purified and identified as *F. oxysporum pv. vasinfectum* is based on its colony color, size, spore shape, structure, and growth pattern. These findings established *F. oxysporum pv. vasinfectum* is the responsible pathogen for wilt disease in okra. Additionally, the research described the symptoms of wilt in infected okra plants, including plant collapse, drooping, wilting, root discoloration, and smaller, discolored seeds. Figures were provided to illustrate the symptoms and the effects of *F. oxysporum pv. vasinfectum* application. Isolation of the pathogen from okra seed is shown in Fig. 1.

The results showed that the associated pathogen of wilt disease has sickle shape microconidia. Based on plate growing pattern, fungal growth speed, spore type and structure clarify that the *F. oxysporum pv. vasinfectum* is responsible for wilt disease. After isolation and identification

of the fungal pathogen associated with disease, an infection test was performed on the potted plants to decide the status of isolated pathogen as established pathogen of the plant. After infection the seedlings collapsed, laid down on ground and become dull brownish or wilted. Mature plants show symptoms of typical drooping along with wilt. Wilted plant roots do not show any external rooting. White to pink coloration due to fungal attack was seen, when split open vertically. On wilted plants fruiting was earlier than treated plants and the seeds of wilted plants were smaller and discolored. Fig. 2 shows the symptoms of wilt on *A. esculentus* by the application of *F. oxysporum pv. vasinfectum.*

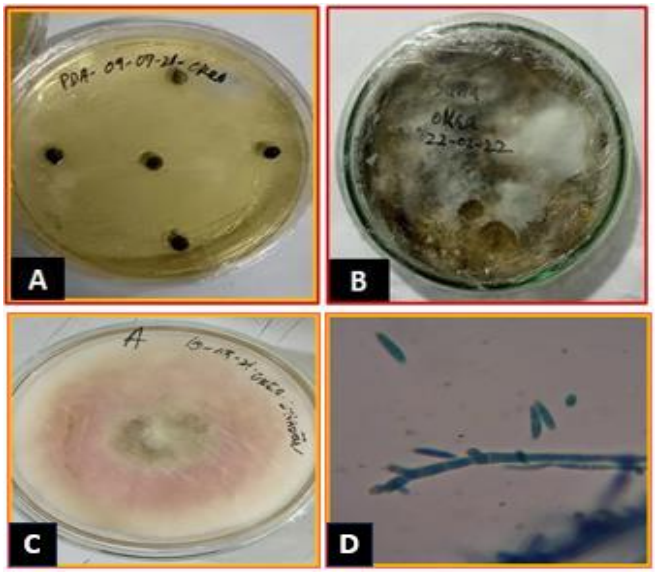

Figure 1. Isolation and purification of pathogen from okra seed whereas A is the placement of samples on PDA plates for isolation of Fusarium, B and C are the pure culture for two isolates of the fungus and D presents the stained spores of the fungus under microscope at 40X.

Figure 2. Symptoms of wilt on *A. esculentus*. Plants showing symptoms of wilt by the infection of *F. oxysporum* pv. *vasinfectum*.

***Molecular characterization via ITS amplification*:** A conventional PCR protocol was employed for the identification of the ribosomal gene as a diagnostic method to selectively amplify *F. oxysporum pv. vasinfectum.* The identification of *F. oxysporum pv. vasinfectum* was based on the generated fragment size, and the presence of the pathogen in symptomatic plants was confirmed by comparing the size of the PCR product with a 1 kb marker. Molecular characterization of the isolated sample was performed by amplifying the 18S ribosomal gene using ITS-1 and ITS-4 primer pairs. The DNA bands obtained from the PCR amplification were visualized using a Gel doc transilluminator under UV light after electrophoresis on a 1% agarose gel. The amplification of isolates processed for genomic DNA from *F. oxysporum pv. vasinfectum*, was observed in the amplified samples. The results were presented in Figure 3, illustrating the amplified samples on the agarose gel, with the 1 kb marker and the isolates' amplification.

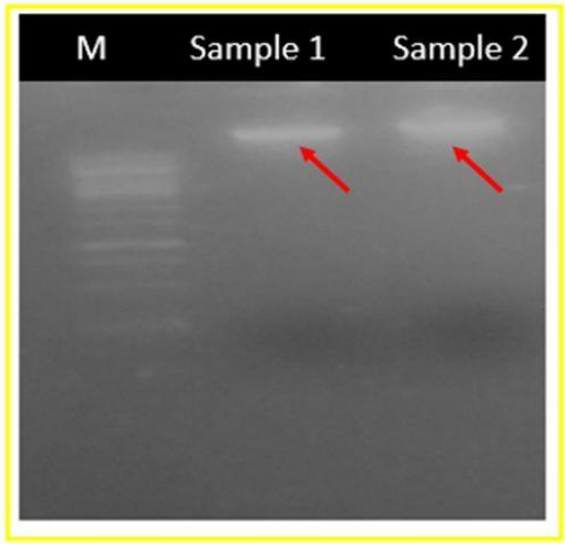

Figure 3. DNA Extraction from *F. oxysporum pv. vasinfectum* using a Modified CTAB Method. Agarose gel (1%) electrophoresis was performed at 80V for 30 minutes.

The DNA samples analyzed include M (1 kb marker) and Sample 1 and Sample 2, representing isolates processed for genomic DNA of *F. oxysporum pv. vasinfectum*.

A conventional PCR protocol was performed under standard conditions to amplify the ITS encoding gene as a diagnostic method for selective amplification and identification of *F. oxysporum pv. vasinfectum* in okra. Through PCR amplification, the presence of *F. oxysporum pv. vasinfectum* was successfully detected in symptomatic plants. Figure 4 displays the amplification of the ITS region using ITS-1 and ITS-4 primers, followed by running the PCR product on a 1% agarose gel. Electrophoresis was performed at 80 V for 30

minutes. The results were visualized on the gel, with the 1 kb marker and the PCR product of *F. oxysporum pv. vasinfectum* positioned at the fourth position.

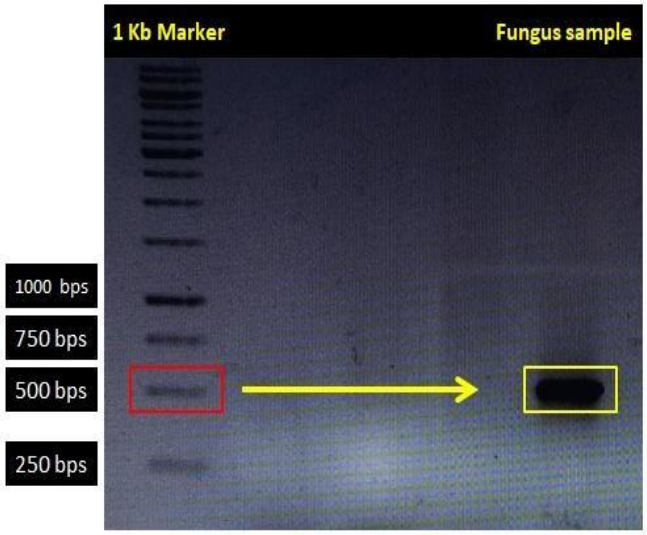

Figure 4. ITS rDNA PCR amplification products using forward primer ITS1 and reverse primer ITS4. ITS region amplification products with ITS-1 and ITS-4 using 1 % agarose gel. Electrophoresis conducted at a voltage of 80V for a duration of 30 minutes. M is 1 kb marker while in the 4th position are PCR product of *F. oxysporum* pv. *vasinfectum* respectively.

***Effect of bio-control agents on F. oxysporum and plant growth parameters*:** Two BCAs, *T. harzianum* (an antagonistic fungus) and *Bacillus spp.* (a bacterium), were used in the study. The antagonistic interactions yielded varying responses from the BCA fungi. Compared to the control, both the antagonistic fungus and bacteria significantly inhibited the growth of *F. oxysporum pv. vasinfectum.* Among the tested bio-control microorganisms, *B. subtilis* exhibited the highest mycelial growth inhibition, accounting for 41.2% of the total inhibition. *T. harzianum*, on the other hand, inhibited *F. oxysporum pv. vasinfectum* by 38.5%. Significant differences in growth rate were observed for the *F. oxysporum pv. vasinfectum* pathogen across different control groups.

Upon application of the BCA, the plants treated under control conditions exhibited the maximum height. In contrast, the plants treated with the pathogen without the use of BCA showed reduced plant height. Fig. 5 visually represents the management of the okra trial in pots, highlighting the effects of treating the plants with *B. subtilis*, *T. harzianum*, and untreated control plants.

***Graphical representation of the effects of BCAs on wilt disease of Okra*:** The graphical representation of the results provides visual evidence of the effects of different treatments and disease conditions on plant growth and disease management. Fig. 6 demonstrates the absence of any interaction among the genotypes of the plant, indicating their independence and significant differences from each other. This finding is consistent with the statistical analysis conducted using ANOVA. It also depicts the interactions between different BCA treatments and two genotypes of okra in pot trials. The control group exhibited the maximum plant height, while the application of *Bacillus* resulted in higher plant height compared to *T. harzianum* treatment. The negative control, which was not treated with any BCA, showed the minimum plant height.

Figure 5. Management trial of okra in the earthen pots, treated with *B. substilis, T. harzianum* and untreated control plants.

Furthermore, the graph shows the interaction between disease conditions and BCA treatments on the root length of Okra plants of genotypes Sabz Pari and Hybrid. The control group displayed the maximum root length. After the application of *Bacillus spp.*, there was a significant increase in root length, followed by *T. harzianum* treatment. In contrast, the negative control exhibited a decreasing trend in root length. It presents the interaction between disease conditions and BCA treatments on the shoot length of genotypes Sabz Pari and Hybrid. The control group demonstrated the maximum shoot length. There was a significant increase in shoot length, followed by *T. harzianum* treatment. The negative control showed the minimum shoot length. Figure showcases the interaction between disease conditions and BCA treatments on disease incidence in plants of genotypes Sabz Pari and Hybrid. The control group exhibited the highest disease incidence, which was reduced after the application of BCA during the experiment. *Bacillus spp.* Treatment resulted in maximum disease control, while Trichoderma exhibited less

efficiency. The control plants had a higher disease incidence, while the negative control showed traces of the disease.

Graphs display the interactions between disease conditions and BCA treatments on various parameters such as dry shoot weight, dry root weight, dry plant weight, fresh plant weight, fresh root weight, and fresh shoot weight, respectively. After the application of *Bacillus spp.*, there was a significant increase in the parameter, followed by *T. harzianum* treatment. The negative control showed minimal or decreased values for these traits. These graphical representations provide a comprehensive overview of the outcomes of the experimental study, emphasizing the influence of different treatments and disease conditions on plant growth, disease incidence, and biomass accumulation.

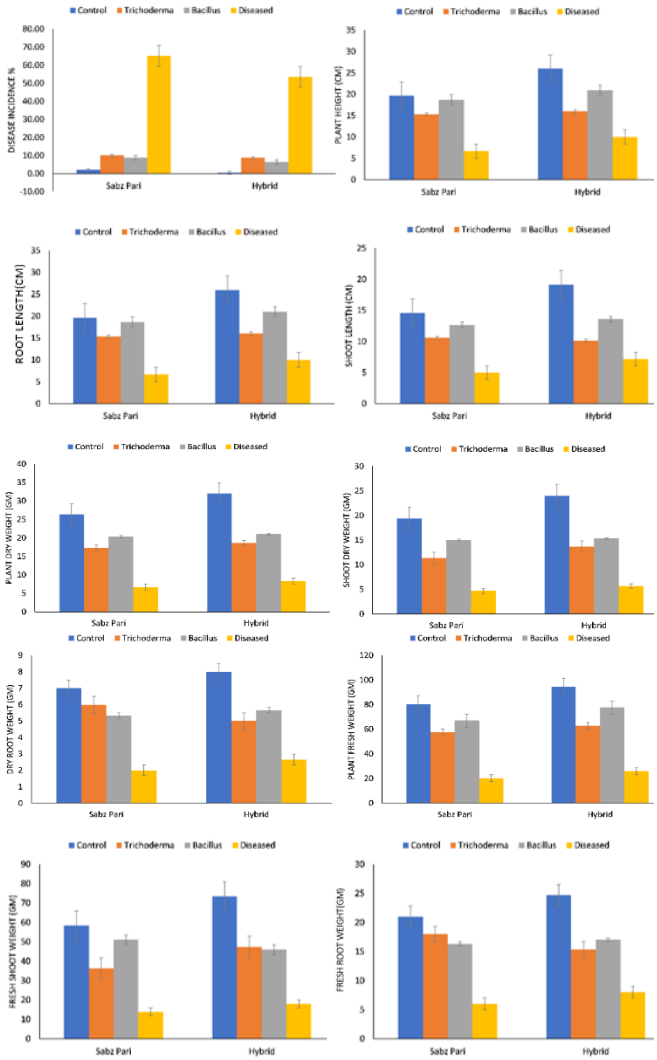

Figure 6. Effects of BCAs *T. harzianum* and *Bacillus spp.* Treatments on the performance of disease incidence, plant height, root length, shoot length, plant dry weight, shoot dry weight, root dry weight, plant fresh weight and root fresh wight for growth parameters against Fusarium wilt in Okra.

DISCUSSION

Fusarium wilt, a highly damaging disease affecting Okra plants worldwide, has been the focus of extensive research (Ismaila *et al.*, 2023). Numerous fungicides have been tested in both *in-vivo* and *in-vitro* studies against the causative agent, *F. oxysporum* (Attia *et al.*, 2021). *F. oxysporum pv. vasinfectum* penetrates the root and stem tissues, blocking the water transport pathways and resulting in the yellowing and desiccation of leaves, ultimately leading to plant dying (Farias *et al.*, 2019). However, the high application rates and hazardous consequences associated with chemical pesticides have impelled a modification towards the use of antagonistic organisms and environmentally friendly plant-based biodegradable products as effective alternatives to combat plant diseases (Khursheed *et al.*, 2022).

The current study explores the significance of the okra plant (*A. esculentus*), highlighting its global cultivation due to low production costs. It focuses on *F. oxysporum* as a fungal pathogen responsible for wilt and damping-off in okra plants, specifically identified as *F. oxysporum f. sp. vasinfectum*. The experiment was conducted using pot cultivation, with four groups established in three replications. The negative control group solely received disease inoculum, while the other groups were treated with fungal inoculum followed by the application of different biocontrol agents (BCAs) at specific intervals. The present experiment also deliberates the isolation and identification of the pathogen through microscopy and PCR techniques, followed by its purification and multiplication. The internal transcribed spacer (ITS) region of the Fusarium isolate was amplified using universal primers ITS1 and ITS4. Subsequent agarose gel electrophoresis of the PCR-amplified DNA confirmed that the chosen primer pair specifically amplified the expected 18S rDNA band, which was 500 bp in size.

These results showed a significant amplification of the targeted gene in the test samples compared to the negative control, confirming the successful purification and identification of the gene. Many scientists have been using universal primers (ITS1 & ITS4) and identified *F. oxysporum* by PCR technique in different crops (Sharma *et al.*, 2018; Agbaglo *et al.*, 2020; Achari *et al.*, 2023). Further, the study emphasizes the importance of using BCAs for disease control, as antagonistic organisms have proven effective in not only combating plant pathogens but also enhancing agricultural crop production. Various species of antagonistic fungi, such as *T. harzianum*, Gliocladium, Paecilomyces, Penicillium, and Verticillium, have demonstrated satisfactory control of destructive plant diseases (Panth *et al.*, 2020; Nysanth *et al.*, 2022; Huilgol *et al.*, 2022). The present experiment indicates

the effectiveness of specific BCAs in inhibiting *F. oxysporum* growth, increasing root and shoot growth, and reducing plant mortality. In the literature, the application of BCAs, including *T. harzianum*, *P. variotii*, *G. virens*, *P. lilacinus*, *B. subtilis*, and *Streptomyces sp.*, has successfully controlled *F. oxysporum* infections in okra, chickpea, and soybean crops (Shah *et al.*, 2015; Maitlo *et al.*, 2019).

Moreover, the findings from the current study reveal that BCAs are effective in controlling Fusarium wilt in okra, as plants treated with Bacillus exhibit improved resistance and vigor against the disease. *In-vitro* tests reveal that *Bacillus sp.* shows a higher percentage (41.2%) of mycelial growth inhibition compared to Trichoderma (38.5%) against *F. oxysporum* pv. *vasinfectum*. Ther performance edge of *Bacillus sp.* is due to their competitiveness in natural niche to colonize. These findings indicate the significant control of pathogen growth achieved with the application of antagonists compared to the uncontrolled growth observed in the control group. (Roy *et al.*, 2015; Zaim *et al.*, 2018).

Furthermore, the interactions between treatments and genotypes of okra were analyzed. It was observed that in the control conditions, the plants exhibited maximum height, root length, shoot length, dry shoot weight, dry root weight, dry plant weight, fresh plant weight, fresh root weight, and fresh shoot weight. Similarly, these results also seem to support with prior research, showing characteristics that are consistent with improved growth parameters. (Chowdappa *et al.*, 2013; Ali *et al.*, 2017). After the application of *Bacillus sp.*, there was a significant increase in these parameters, followed by Trichoderma treatment.

However, the negative control group showed minimal or decreased values for these traits. These findings demonstrate the positive influence of BCAs on plant growth and disease management. The use of BCAs, particularly Bacillus, not only reduced disease incidence but also resulted in improved morphological traits and biomass accumulation. This highlights the potential of bio-control strategies for enhancing the vigor and productivity of host plants, providing them with better resistance against multiple pathogens. The results suggest that the application of BCAs, such as *T. harzianum* and *Bacillus sp.*, can effectively control the target pathogen and promote the growth and development of the host plant.

***Conclusion*:** Bacillus and Trichoderma were found to be effective in controlling the disease, exhibiting significant inhibition of the pathogenic fungus Fusarium. The highest level of control was achieved with *Bacillus sp.*, which demonstrated a 41.2% inhibition, followed by Trichoderma with a 38.5% inhibition of *F. oxysporum pv. vasinfectum*. The identified BCAs were further tested in laboratory and field conditions, leading to the selection of the most promising agents for disease management. Given the detrimental impact of *F. oxysporum pv. vasinfectum* on the root tissues of Okra, it is important to adopt environmentally friendly solutions for disease control, as excessive use of chemicals can negatively affect the quality of the okra vegetable. The results from this study provide valuable insights into the safe and effective management of wilt disease in Okra using BCAs, both in laboratory tests and field applications. This research contributes to the development of sustainable agricultural practices for the cultivation of this important vegetable crop. Additional research is required to explore the efficacy of BCAs and assess the feasibility of utilizing the specific microbes for effectively suppressing pathogens.

***Authors' contributions*:** Syeda Sana Fatima and Muhammad Nabeel Afzal conducted research, Amjad Abbas and Muhammad Amjad Ali planned and did write up while Muhammad Huzaifa Tanveer, and Muhammad Jabran helped in research while Shahneeza Aimal Nadeem helped in final manuscript.

***Conflict of interest*:** The authors declare no conflict of interest.

***Acknowledgement*:** We are thankful to Dr. M. Naveed for providing BCA strains

***Funding*:** The authors acknowledge Higher Education Commission for providing research funding through Project No. NRPU-6444.

***Ethical statement*:** This article does not contain any studies regarding human or Animal.

***Availability of data and material*:** We declare that the work presented in the manuscript is our own work, which has not been published before and is not currently being considered for publication elsewhere.

***Code availability*:** Not applicable. Consent to participate: All authors participated in this research study.

***Consent for publication*:** All authors submitted consent to publish this research article

REFERENCES

Achari, S. R. Mann, R.C. Sharma and M.J Edwards. 2023. Diagnosis of Fusarium oxysporum f. sp. ciceris causing Fusarium wilt of chickpea using loop-mediated isothermal amplification (LAMP) and conventional end-point PCR. Scientific Reports 13:2640.

Agbaglo, S.Y., S.T. Nyaku, H.D. Vigbedor and E.W. Cornelius. 2020. Pathogenicity of Meloidogyne incognita and Fusarium oxysporum f. sp. vasinfectum on growth and yield of two okra varieties cultivated in ghana. International Journal of Agronomy 1-12.

Ali, A. A., A.E. Abd El-Kader and K.M. Ghoneem. 2017. Two Trichoderma species and Bacillus subtilis as biocontrol agents against Rhizoctonia disease and their influence on potato productivity. Egyptian Journal of Agricultural Research 95:527-541.

AL-Taae, W. and A.K. Al-Taae. 2019. First molecular identification of Fusarium oxysporum causing Fusarium wilt of Armenian cucumber in Iraq, IOP Conference

Series: Earth and Environmental Science. IOP Publishing, Bristol, UK 338: 1-6.

Ashraf, M.R., O.B.A. Hafeez, A. Masroor, S. Ahmad and I. Zaib. 2018. Okra Seeds Priming with Two Bacilli *sp.sp.* and TriHar-6 Isolates Enhanced the Physiological Attributes and Suppressed the Fusarium oxysporum f. sp. vasinfectum. Annals of the Romanian Society for Cell Biology 34-49.

Attia, M. S., H.A. El-Naggar, M.M. Abdel-Daim and G.S. El-Sayyad. 2021. The potential impact of Octopus cyanea extracts to improve eggplant resistance against Fusarium-wilt disease: in vivo and in vitro studies. Environmental Science and Pollution Research 28: 35854-35869.

Banerjee, S., F. Walder, L. Büchi, M. Meyer, A.Y. Held, A. Gattinger and M.G.Van der Heijden. 2019. Agricultural intensification reduces microbial network complexity and the abundance of keystone taxa in roots. The ISME journal 13:1722-1736.

Chowdappa, P., S.M Kumar, M.J. Lakshmi and K. K. Upreti. 2013. Growth stimulation and induction of systemic resistance in tomato against early and late blight by Bacillus subtilis OTPB1 or Trichoderma harzianum OTPB3. Biological control 65:109-117.

Farias, O.R., L.C. Nascimento, J.M.F. Lima, H.A.O. Silva, M.D. Mello, L.A. Bruno and N.H.C. Arriel. 2019. Biocontrol potential of Trichoderma and Bacillus species on Fusarium oxysporum f.sp vasinfectum. Journal of Experimental Agriculture International 34:1-11.

Huilgol, S. N., K.L. Nandeesha and H. Banu. 2022. Fungal biocontrol agents: an eco-friendly option for the management of plant diseases to attain sustainable agriculture in India. In Fungal diversity, ecology and control management Singapore: Springer Nature Singapore. pp. 455-481.

Ismaila, A.A., K. Ahmad, Y. Siddique, M. A. A. Wahab, A.B. Kutawa, A. Abdullahi and S.N.A. Abdullah. 2023. Fusarium wilt of banana: Current update and sustainable disease control using classical and essential oils approaches. Horticultural Plant Journal 9:1-28.

Khursheed, A., M.A Rather, V. Jain, S. Rasool, R. Nazir, N. A. Malik and S.A. Majid. 2022. Plant based natural products as potential ecofriendly and safer biopesticides: A comprehensive overview of their advantages over conventional pesticides, limitations and regulatory aspects. Microbial Pathogenesis, 105854.

Kipngeno, P., T. Losenge, N. Maina, E. Kahangi and P. Juma.2015. Efficacy of Bacillus subtilis and Trichoderma asperellum against Pythium aphanidermatum in tomatoes. Biological Control 90:92-95.

Kumar, S. D. 2019. Enumerations on seed-borne and post-harvest microflora associated with okra [Abelmoschus esculentus (L.) Moench] and their management. *GSC* Biological and Pharmaceutical Sciences 8:

Lahlali, R., S. Ezrari, N. Radouane, J. Kenfaoui, Q. Esmaeel, H. El Hamss and E. A. Barka. 2022. Biological control of plant pathogens: A global perspective. Microorganisms 10: 596.

Ma, Z., Z. Guan, Q. Liu, Y. Hu, L. Liu, B. Wang and M. Saleem. 2023. Obstacles in continuous cropping: mechanisms and control measures. Advances in agronomy 179:205-256.

Samarrai T.H.A. and J. Schmid. 2000. A simple method for extraction of fungal genomic DNA. Letters in Applied Microbiology 30:53-56.

Maitlo, S.A., N.A. Rajput, R.N. Syed, M. A. Khanzada, A.Q. Rajput and A.M. Lodhi. 2019. Microbial control of Fusarium wilt of chickpea caused by *Fusarium oxysporum* f. sp. ciceris. Pakistan Journal of Botany 51:2261-2268.

Nysanth, N.S., S. Divya, C.B. Nair, A.B. Anju, R. Praveena and K.N. Anith. 2022. Biological control of foot rot (Phytophthora capsici Leonian) disease in black pepper (Piper nigrum L.) with rhizospheric microorganisms. Rhizosphere 100578.

Ons, L., D. Bylemans, K. Thevissen and B.P. Cammue. 2020. Combining biocontrol agents with chemical fungicides for integrated plant fungal disease control. Microorganisms 8: 1930.

Panth, M., S.C. Hassler and F. Baysal-Gurel. 2020. Methods for management of soilborne diseases in crop production. Agriculture 10:16.

Roy, S., A. Rathod and A. Pramanik. 2015. Bioefficacy of Bacillus subtilis against root knot nematode Meloidogyne incognita (Kofoid and White) Chitwood in tomato. Journal of Applied and Natural Science 7:1012-1015.

Shah, N., R.N. Syed, M.A. Khanzada, N.A. Rajput and A.M. Lodhi. 2015. Non-chemical management of okra wilt caused by Fusarium oxysporum. International Journal of Agricultural and Applied Sciences *7*: 86-94.

Sharma, K., D. Hemlata, R. Rathour, R.K. Kapila and Y.S. Paul. 2018. Detection of pea wilt pathogen Fusarium oxysporum f. sp. pisi using DNA-based markers. Journal of Plant Biochemistry and Biotechnology 27:342-350.

Tucho, A. and G. Yadessa. 2014. Distribution and Occurrence of Mango Anthracnose (Colletotrichum gloesporioides Penz and Sacc) in Humid Agro-ecology of Southwest Ethiopia. Plant Pathology Journal 13:268-277. 10.3923/ppj.2014.268.277.

Xie, M., Z. Yan, X. Ren, X. Li and B. Qin. 2017. Codonopilate A, a triterpenyl ester as main autotoxin in cultivated soil of Codonopsis pilosula (Franch.) Nannf. Journal of Agricultural and Food Chemistry 65:2032-2038.

Zaim, S., A.A. Bekkar and L. Belabid. 2018. Efficacy of Bacillus subtilis and Trichoderma harzianum combination on chickpea Fusarium wilt caused by F. oxysporum f. sp. ciceris. Archives of Phytopathology and Plant Protection 51:217-226.